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Construction of a Functional Disulfide-Stabilized TCR Fv Indicates that Antibody and TCR Fv Frameworks Are Very Similar in Structure

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Summary

Disulfide-stabilized Fvs (dsFv) are recombinant Fv fragments of antibodies in which the inherently unstable V_H–V_L heterodimer is stabilized by an interchain disulfide bond engineered between structurally conserved framework positions. We now design and produce a disulfide-stabilized Fv of a T cell receptor. It is composed of Vα and Vβ variable domains of the 2B4 TCR stabilized by a disulfide bond between framework residues of the TCR Fv at a site corresponding to that used for disulfide stabilization of antibody Fvs. For ease of production and detection, the TCRdsFv was fused to a truncated form of Pseudomonas exotoxin (PE38). The TCR(dsFv) retains its native conformation and is much more stable than a TCR scFv. Moreover, it is functional in biological assays. Because successful disulfide stabilization of the TCR Fv by the positions used for antibody Fv stabilization would not occur unless the mutated residues in TCR Fv are at positions closely similar to those in antibody Fvs, most likely within less than 1.5 Å, these results provide very strong experimental evidence for the structural similarity between immunoglobulin and TCR antigen-binding variable domains.

Introduction

The T cell antigen receptor (TCR) is a membrane-bound receptor composed of two chains, α and β, each consisting of an N-terminal variable region (V) and a C-terminal constant region (C) (Davis and Bjorkman, 1988; Chothia et al., 1988; Novotny et al., 1986; Claverie et al., 1989). Analysis of the sequences of the TCR chains indicate that they have a framework closely related to that of immunoglobulins (Davis and Bjorkman, 1988; Chothia et al., 1988; Kronenberg et al., 1986). Further comparisons of the V chains of TCRs with V regions of immunoglobulin Gs (IgGs) indicated TCR variable domains have four hypervariable regions, three of which can be aligned with the complementarity-determining regions (CDR) of IgGs to produce a Fab-like model of the TCR (Davis and Bjorkman, 1988; Chothia et al., 1988; Kronenberg et al., 1986; Jores et al., 1990). Thus, it was proposed that the three-dimensional structure of TCR should be similar to the structure of antibodies (Davis and Bjorkman, 1988; Chothia et al., 1988; Novotny et al., 1986; Claverie et al., 1989; Kronenberg et al., 1986; Jores et al., 1990). However, the three-dimensional structure of the TCR αβ heterodimer has not yet been determined, in part because crystallography requires the production of relatively large amounts of a soluble form of this complex. Isolation of the soluble αβ heterodimer is difficult because the α and β chains are both type transmembrane proteins and are not soluble in the absence of detergent (Davis and Bjorkman, 1988). To circumvent this problem, several genetic engineering–based approaches have been used to obtain recombinant soluble TCRs in significant quantities. These include the production of TCR chains truncated prior to the transmembrane domain (Gascoigne, 1990), hybrid molecules composed of the TCR and the constant domain of immunoglobulin (Weber et al., 1992; Gregoire et al., 1991), and altered forms of TCR that can be cleaved from the plasma membrane (Lin et al., 1990; Engel et al., 1992). The success in the expression of single-chain antibody variable domains in Escherichia coli (Huston et al., 1988, 1991; Bird et al., 1988) and the postulated structural similarities between IgGs and TCRs led to the construction and expression of the TCR variable domains as a single-chain molecule (scFv) in which Vα and Vβ are connected by a peptide linker (Novotny et al., 1991; Soo Hoo et al., 1992; Ward, 1992). Recently, a TCR scFv derived from the TCR of the 2B4 T cell hybridoma specific for the C-terminal peptide of cytochrome C bound to I-E^k was produced (Kurucz et al., 1993). The TCRscFv is indistinguishable antigenically from the V region of the native TCR and has binding activity to cells expressing MHC–peptide complexes. One disadvantage of scTCRs as well as antibody scFvs is the difficulty producing these proteins in large quantities and at high concentrations because of their tendency to aggregate (Buchner et al., 1992). To overcome the instability of scFvs, we and others have stabilized scFvs by insertion of a disulfide bond between V_H and V_L (Brinkmann et al., 1993; Reiter et al., 1994a; Jung et al., 1994; Glockshuber et al., 1990). In these disulfide-stabilized Fvs (dsFv), the peptide linker connecting V_H and V_L is replaced by an interchain disulfide bond engineered into conserved framework regions of the V_H and V_L domains of the antibody Fv. The positions for the cysteine replacement in antibody dsFvs are located in conserved framework regions and were used to stabilize various Fvs. The location of the cysteines can be identified by a simple sequence homology alignment and without further structural information or modeling. Since it has been proposed that Fvs of antibodies and TCRs have similar structures, we reasoned that it should be possible to use positions in the TCR sequence that are homologous to the positions identified for disulfide stabilization of antibodies Fvs to make a TCR dsFv. The production of such TCRdsFvs would be important for two reasons: it should be soluble and stable and enable the production of the large amounts required for structural analysis; and if disulfide stabilization using the same positions that stabilize antibody Fvs is success-

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          ----------FR1---------- -------CDR1------ ----FR2----
603(VL)  DIVMTQSPSSLSVSAGERVTMSC KSSQSLLNSGNQKNFLA WYQQKPGQPPK
 B3(VL)  DVLMTQSPLSLPVSLGDQASISC RSSQIIVHS.NGNTYLE WYLQKPGQSPK
2B4(Vβ)  NSKVIQTPRYLVKGQGQKAKMRC IPEKG.......HPVVF WYQQNKNNEFK
                                                   *

603(VL)  LLIY ...GASTRES GVPDR..FTGSGSGTDFTLTISSVQAEDLAVYYC
 B3(VL)  LLIY ...KVSNRFS GVPDR..FSGSGSGTDFTLKISRVEAEDLGVYYC
2B4(Vβ)  FLIN FQNQEVLQQI DMTEKRFSAECPSNSPCSLEIQSSEAGDSALYLC
         ---- ---CDR2--- ---------------FR3----------------

603(VL)  QNDHSYPLT... FGAGTK
 B3(VL)  FQGSHVPFT... FGSGTK
2B4(Vβ)  ASSLNWSQDTQY FGPGTR
         ----CDR3---- -FR4--
                      Δ
          --------------FR1------------- -CDR1 -------FR2------
603(VH)  EVNLVESGGGLVQPGGSLRLSCATSGFTFS DFYME WVRQPPGKRLE..WIA
 B3(VH)  DVKLVESGGGLVQPGGSLKLSCATSGFTFS DYYMY WVRQTPEKRLE..WVA
2B4(Vα)  QVEQSPSALSLHEGTGSALR.CNFTTTM.. .RAVQ WFQQNSRGSLINLFYI
                                                        Δ
603(VH)  ASRNKGNKYTTEYSASVKG RFIVSRDTSQSILYLQMNALRAEDTAIYYCAR
 B3(VH)  YISN..DDSSAAYSDTVKG RFTISRDNARNTLYLQMSRLKSEDTAIYSCAR
2B4(Vα)  ASGTKEN..........G RLKSTFNSKESYSTLHIRDAQLEDSGTYFCAA
         --------CDR2------- --------------FR3--------------

603(VH)  NYYGSTWYFDV WGAGTTVTVSS
 B3(VH)  G.LAWGAWFAY WGQGTLVTVSS
2B4(Vα)  LRVTGGNNKLT FGQGTVLSVIP
         ----CDR3--- ----FR4----
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Figure 1. Disulfide Connection Between Vα and Vβ of 2B4 TCR Fv

Comparison of the variable domains of MAb B3 and 2B4 TCR. Asterisk and open triange, position of cysteine replacements in framework regions of the TCR(Fv) (Ser-43 in Vα and Pro-107 in Vβ, or Gln-104 in Vα and Glu-42 in Vβ). The assignment of framework regions 1–4 (FR1–4) and CDR1–3 is according to Kabat et al. (1991).

ful, it would show that those positions are closely similar to the equivalent positions in antibody Fvs, probably within less than a 1.5 Å discrepancy from the same positions in antibody Fv. This would constitute direct experimental evidence for the structural identity of antibody and TCR Fv framework regions.

Results

Design of Disulfide Connection Between Vα and Vβ of 2B4 TCR Fv

The two possible positions for disulfide stabilization of antibody Fvs (Reiter et al., 1994a; Jung et al., 1994) are V_H-44 and V_L-100 and V_H-105 and V_L-43 (Kabat et al., 1991). These two pairs of positions are strictly in the framework region of the Fv, distant from the CDRs and with a short Cα–Cα distance. We computed the Cα–Cα distances at these sites for many Fv regions for which a crystal structure is known. These data indicate that this short Cα–Cα distance exists for most Fvs and is indeed suitably short for the formation of a disulfide bond at the selected sites (Jung et al., 1994). To identify the corresponding positions in the TCR Fv, we aligned the amino acid sequence of antibodies McPC603, J539, and B3 Fv with the 2B4 TCR Fv (Figure 1). We used the BESTFIT sequence alignment program in the Genetics Computer Group package (Devereux et al., 1984) and visual inspection to align these sequences. When the Vα sequence of 2B4 was aligned to the V_H sequence, the residue corresponding to the V_H-44 site of B3 was identified as Vα-43S (TCR 42 in the numbering scheme of Kabat et al., 1991; the V_H-105 site of B3 was identified as Vα-104Q [TCR 108 in Kabat et al., 1991]). Similarly, the 2B4 residues Vβ 107P and Vβ 42E (TCRs

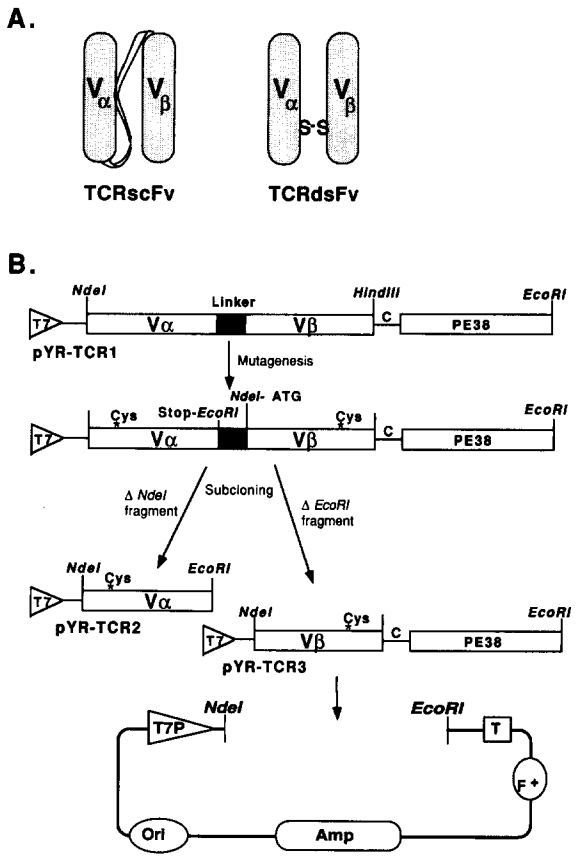

Figure 2. Construction of Plasmids for Expression and Production of TCR(dsFv)–PE38

(A) Schematic representation of TCR scFv and TCR dsFv. In the TCR dsFv, the $(Gly_4\text{-}Ser)_3$ peptide linker is replaced by the designed interchain disulfide bond.

(B) Plasmids for expression of TCR(dsFv)–PE38. Single-stranded uracil-containing DNA of pYR-TCR1 was the template to mutate the identified residues to cysteines by mutagenesis. The expression plasmid pYR-TCR2 for TCR(Vα-Cys) was generated by deletion of a Vβ–PE38-encoding EcoRI fragment. pYR-TCR3-encoding TCR(Vβ-Cys)–PE38 was constructed by deletion of a Vα-encoding NdeI fragment.

110 and 42 in Kabat et al., 1991) were aligned to antibody residues corresponding to V_L-100 and V_L-43 of B3, respectively. We identified the same residues when we aligned the Vα of 2B4 with the V_L of McPC603, and Vβ of 2B4 with the V_H of McPC603, or when we used the sequence of MAb J539 instead of McPC603 for alignment of the 2B4 sequence. Thus, the potential interchain disulfide bond sites are Vα43–Vβ107 and Vα104–Vβ42.

Construction of Plasmids for Expression and Production of Disulfide-Stabilized TCR Fv-PE38

dsFvs are produced as two separate components, V_H and V_L or Vα and Vβ, respectively, which are encoded by separate plasmids and expressed separately (Brinkmann et al., 1993; Reiter et al., 1994a). The plasmids, coding for (Cys-104)Vα and (Cys-42)Vβ-PE38 were made by site-directed mutagenesis and subcloning from TCRscFv-coding plasmid (Figure 2B). In the single-chain molecule,

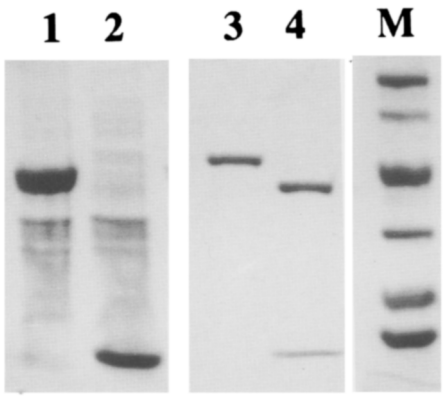

Figure 3. SDS–PAGE Analysis on Reducing Gel of the Disulfide-Stabilized TCR Fv–PE38

Lane M, molecular mass standard; lane 1, IBs containing TCR(Vβ-Cys)–PE38; lane 2, TCR(Vα-Cys); lane 3, purified TCR(scFv)–PE38; lane 4, purified TCR(dsFv)–PE38. Molecular mass standards are (top to bottom) 106, 80, 49, 32, 27, and 18 kDa, respectively. The gel was stained by Coomassie blue.

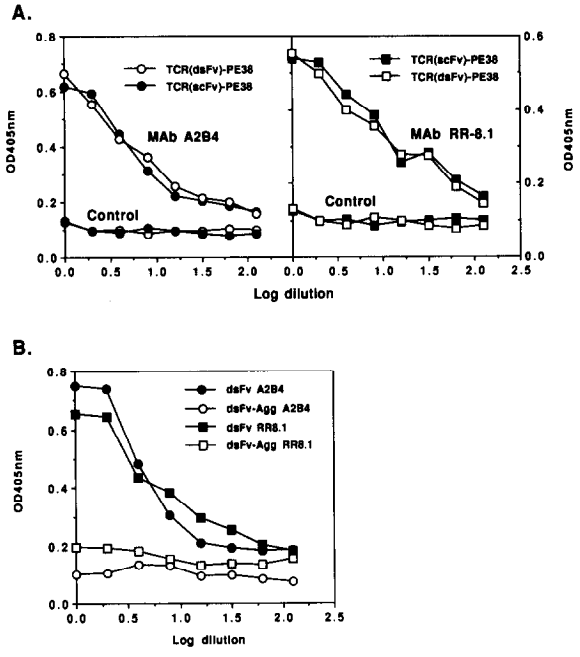

Figure 4. Binding of Anti-TCR MAbs to the 2B4 TCR(dsFv)–PE38 and TCR(scFv)–PE38

The fusion protein were immobilized on 96-well microtiter plates, blocked with 1% bovine serum albumin in PBS, and then incubated with TCR-specific MAbs. The binding was detected by secondary alkaline phosphatase–labeled antibody as described (Kurucz et al., 1993). The 2B4 TCR contains Vα11 and Vβ3 allotypic determinants (Kurucz et al., 1993). A, binding of MAbs to monomeric soluble TCR(dsFv)–PE38 and TCR(scFv)–PE38. B, binding of MAbs to aggregated TCR(dsFv)–PE38.

the Vα and Vβ domains of the TCR Fv are produced as a single molecule, in which the domains are held together by a $(Gly_4\text{-}Ser)_3$ peptide linker. In addition to placing the cysteine mutations in the TCR Fv, we fused a truncated form of Pseudomonas exotoxin (PE38) (Brinkmann et al., 1993) to the TCR Fv for ease of purification and to facilitate subsequent analysis. The TCR(dsFv)–PE38 was expressed in intracellular inclusion bodies, and was refolded in a redox-shuffling system like antibody dsFv fusions (Brinkmann et al., 1993; Reiter et al., 1994a; Figure 3). The properly folded TCR(dsFv) fusion was then purified to near homogeneity by ion-exchange chromatography (Q–Sepharose and Mono Q) followed by size-exclusion chromatography (Figure 3). Using this refolding and purification protocol, we obtained ~20 mg of purified monomeric TCR(dsFv)–PE38 from 1 l of bacterial culture induced at OD_{600} = 9. From this 1 l, approximately 350 mg of protein was present in inclusion bodies, representing a yield of 6%.

SDS–gel analysis of the purified monomeric TCR(dsFv)–PE38 protein (Figure 3) indicates homogenous molecules composed of only α:β heterodimer with apparent molecular mass of 62 kDa as expected. Since the PE38 moiety (38 kDa) is fused to the TCR Vβ, formation of β:β or α:α homodimers would result in proteins with an apparent molecular mass of ~100 kDa (for β:β dimers) or ~24 kDa (for α:α dimers), respectively, which were not detected after purification.

The Disulfide-Stabilized TCR Fv Retains its Native Conformation

The disulfide-stabilized TCR(dsFv)–PE38 that we obtained has the native conformation of the TCR Fv by two criteria; first, it reacts with conformation-specific monoclonal antibodies (MAbs) directed to the V domain of TCR. As shown in Figure 4A, the reactivity of TCR(dsFv)–PE38 and TCR(scFv)–PE38 with MAb RR8-1, specific for Vα11, and MAb A2B4, an anti-2B4 idiotype antibody (Kurucz et al., 1993), was identical, while a control antibody of the same isotype class did not bind to the TCR Fv fusion protein. The A2B4 antibody does not bind to reduced and alkylated 2B4 TCRscFv because it recognizes a conformationally dependent epitope (Kurucz et al., 1993). This antibody also failed to bind to improperly folded aggregated forms of TCR(dsFv)–PE38 (Figure 4B). Second, the refolded protein is monomeric in nondenaturing solution (see Figure 7), a good indication that the protein has folded correctly (Buchner et al., 1992). The fact that MAb A2B4 recognizes the correctly folded TCRscFv as well as the dsFv monomers indicates that disulfide stabilization of the TCR Fv did not cause significant structural alterations that would abolish the specific binding of antibodies directed to conformational epitope.

The TCR(dsFv) Is Functional in Cellular Assays

To test whether the TCR(dsFv) is functional, we studied its biological activity in cellular assays in which the inhibition of the antigen-specific activation of a T cell hybridoma is measured. For these assays, the T cell hybridoma, 2B4, specific for the C-terminal peptide of moth cytochrome c bound to $I\text{-}E^k$, was stimulated with suboptimal concentrations of the antigen moth cytochrome c peptide presented by irradiated spleen cells from B10.A mice, in the absence

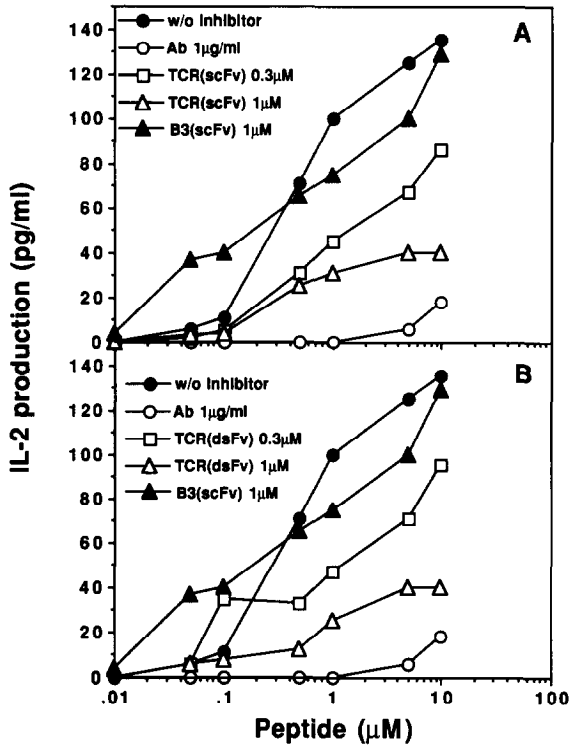

Figure 5. Cellular Inhibition Assays with TCR(dsFv)

The 2B4 T cell hybridoma was stimulated with suboptimal concentrations of antigenic peptide using irradiated spleen cells from B10.A mice as a source for antigen-presenting cells. This was performed in the absence or presence of anti-class II antibody or TCR(dsFv)–PE38. T cell activation was monitored by measuring IL-2 secretion.

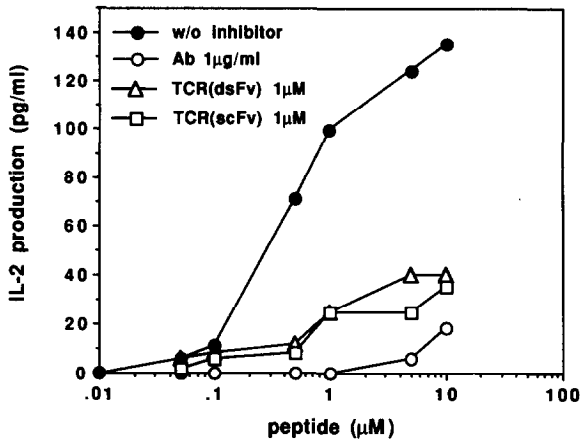

Figure 6. Comparison of the Activity of TCR(dsFv) and TCR(scFv) in Cellular Inhibition Assays

Assays were performed as described in Figure 5. The inhibitors were anti-class II antibody or the TCR(Fv) constructs, TCR(dsFv)–PE38 or TCR(scFv)–PE38.

or presence of the TCR constructs or anti-MHC class II antibody, 14-4-4 (anti I-$E^{k/d}$; Ozato et al., 1980). The antigen-dependent activation or inhibition of the T cell response is measured by the production and secretion of interleukin-2 (IL-2). Figure 5 shows peptide-dependent activation of the 2B4 T cell hybridoma that can be inhibited efficiently by the anti-class II antibodies. The antigen-specific activation was inhibited in a dose-dependent manner by the TCR(dsFv) construct (Figure 5). Efficient inhibition, which was only 2- to 3-fold lower than the inhibition by anti-class II antibody, was obtained at 1 μM of TCR(dsFv)–PE38. The inhibition by the TCR(scFv) and TCR(dsFv) constructs was similar (Figure 6), indicating that both TCR constructs are functional and disulfide stabilization of the TCR(Fv) did not affect its binding capacity as judged by these inhibition assays. A control construct of an anti-carbohydrate antibody Fv, B3(Fv)–PE38-M, did not have any effect on the antigen-specific stimulation of the 2B4 hybridoma. This control excludes the possible toxic effects of the PE38 domain in the TCR(Fv) constructs on the T cell hybridoma or spleen cells, even at the high concentration of 60 μg/ml required for efficient inhibition of the specific T cell response.

Stability of Disulfide-Stabilized TCR Fv–PE38

To test the effect of disulfide stabilization on stability, the TCR(dsFv)–PE38, and the TCR(scFv)–PE38 were incubated at a concentration of 100 μg/ml in phosphate-buffered saline (PBS) at 37°C for various times, and the molecular forms of these molecules determined by their mobility in size-exclusion chromatography (Figure 7). We found that the TCRscFv was unstable, since the monomeric fraction was rapidly converted to aggregates with a higher molecular weight. In contrast, the TCRdsFv–fusion protein showed no aggregates after 1 hr and only a small amount of aggregates after 4 hr. The TCR(scFv)–PE38 aggregated much more than the TCR(dsFv)–PE38 but less than the TCRscFv alone (data not shown). These results demonstrate that properly folded TCR(dsFv)–PE38 molecules have a markedly reduced tendency to aggregate. This finding is in complete agreement with previous observations that disulfide-stabilized Fvs of antibodies are more stable than their single-chain counterparts due to a reduced tendency of dsFv molecules to aggregate (Reiter et al., 1994a, 1994b, 1994c). The cysteine bridge in the dsFv apparently helps to maintain the integrity of the molecule by stabilizing the heterodimer in a correct conformation in which it retains its antigen binding capacity.

Discussion

We have made a functional disulfide-stabilized Fv of a TCR using the same positions that stabilize antibody Fvs. These results show that the positions chosen are closely similar to the equivalent positions in antibody Fvs, and constitute strong direct experimental evidence for the structural identity of antibody and TCR Fv framework regions. The sequence homology between TCR and antibodies is not especially high. TCR α and β domains have an average 25%–30% identity to immunoglobulin V_L and V_H (Claverie et al., 1989). For example, the 2B4 TCR Vα and Vβ domains share only 20%–26% identity and 40%–46% similarity with the V_H and V_L domains of antibody McPC603. However, the invariant side chains defining the

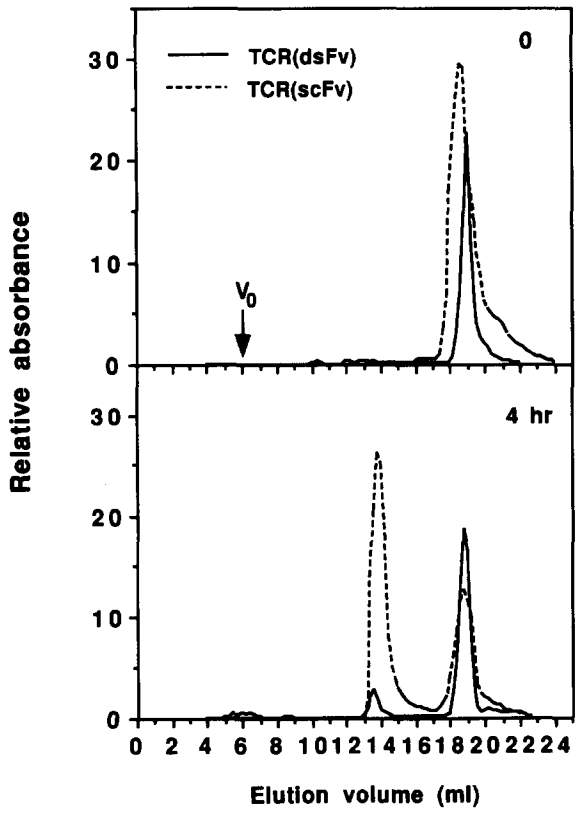

Figure 7. Stability of 2B4 TCR scFv and TCR Fv–PE38 Fusion Proteins

TCR(scFv)–PE38 or TCR(dsFv)–PE38 were incubated at 100 μg/ml in PBS at 37°C for the indicated time. The molecular form of these molecules was then analyzed on a TSK3000 size-exclusion chromatography. Monomers elute at 18–19 ml and aggregates elute at 13–14 ml.

framework of the structure for antibody binding sites are shared with the T cell immunoglobulin-like chains, most notably the αβ receptors (Novotny et al., 1986; Claverie et al., 1989; Hedrick et al., 1984). The justification for expecting a similarity in the three-dimensional structures for these molecules is based on the general rule that in the evolution of multigene families, such as the immunoglobulins, protein-folding motifs are even more conserved than the amino acid sequence (Novotny et al., 1986).

The results of the experiments reported here constitute strong direct experimental confirmation of this generally accepted expectation. The positions used for the disulfide stabilization of the TCR Fv were initially identified for antibody Fvs by computer modeling. Afterwards, the appropriate interchain cysteine positions in the 2B4 TCR Fv were identified by simple alignment of TCR and antibody sequences without any modeling. Therefore, the successful formation of the disulfide bond at this site indicates that at least the two residues involved, and probably all the residues that are equivalent by sequence homology, are located at very similar relative positions in the two structures.

How similar? In the case of the antibody B3 Fv, the Cα–Cα distance between the pair of residues that could be successfully connected by a disulfide bridge is 5.7 Å in the computer-modeled structure (Jung et al., 1994). The corresponding Cα–Cα distances in all known antibody structures range from 4.1–6.9 Å (Jung et al., 1994). If the corresponding Cα–Cα distance in the TCR Fv deviated more than this range, a disulfide bond may not form. Recently, attempts were made to introduce an interchain disulfide bond in B3 Fv at many sites around the site identified by computer modeling. All attempts failed, including one for which the Cα–Cα distance is 6.4 Å in the computer-modeled structure (Y. R., unpublished data). This is reasonable in view of the fact that the Cα–Cα distance between two cysteine residues that form the disulfide bonds of the type likely to form at this site is 5.0 Å on average (Jung et al., 1994; Katz and Kossiakoff, 1986). It can therefore be expected that the mainframe structure of the TCR deviates from that of a typical antibody by less than about 1.5 Å (half of the range of variation in the Cα–Cα distances given above).

The ultimate proof for the structural similarity of Fvs of antibodies and TCRs will only be provided by solving the TCR three-dimensional structure. The possibility of producing large amounts of stable soluble TCR Fv in the form of a dsFv should help achieve this goal. The effect of the truncated Pseudomonas exotoxin in the fusion proteins or the ability to crystalize the protein is not known. However, the improved stability of the TCR(dsFv) and recent results on stability of antibody dsFvs (Reiter et al., 1994b) may enable the production of a TCR(dsFv) alone.

We also show in this study that the TCR(dsFv) produced is functional in cellular assays in which the inhibition of the antigen-specific stimulation of a T cell hybridoma is measured. The TCR(dsFv) can inhibit in a dose-dependent manner T cell stimulation with suboptimal concentrations of antigenic peptide. This example demonstrates the functionality of a TCR(Fv) construct by inhibiting antigen-dependent stimulation of T cells. Such inhibitory activity was demonstrated previously only by TCR–immunoglobulin chimeria with the variable and the first constant regions of both the TCR α and β chains linked to the immunoglobulin light chain constant region (Weber et al., 1992). The inhibition by TCR(dsFv) and TCR(scFv) constructs was similar, indicating that disulfide stabilization of the TCR(Fv) did not affect its MHC binding capacity.

In conclusion, our data provide strong experimental evidence for the structural identity of TCR and immunoglobulin Fv antigen binding domains.

Experimental Procedures

Plasmid Constructions

The parent plasmid for the generation of plasmids for expression of TCR(dsFv)–PE38 encodes the single-chain Fv of the 2B4 TCR in which the TCR Vα and Vβ domains are held together by a $(Gly_4\text{-}Ser)_3$ peptide linker and fused to a truncated form of Pseudomonas exotoxin (Kurucz et al., 1993). The complete nucleotide sequence of 2B4 TCR(Fv) has been described (Kurucz et al., 1993). Uracil-containing single-stranded DNA from the F^+ origin present in this plasmid was obtained by cotransfection with M13 helper phage and was used as template for site-directed mutagenesis as described (Kunkel, 1985). The mutagenic oligonucleotides 5′-AACACTCAGAACGGTACCGCAACCAAAAGT-CAGCTT-3′ and 5′-GAACAGATTGATGAGGCAGCCCCGGGAATTC-

TGTTGGAA-3′ were used to change Gln-104 and Ser-43 of 2B4 TCR Vα to cysteines, respectively. The oligonucleotides 5′-GTTAATCAAA-AATTTGAAGCAATTGTTCTTATTTTG-3′ and 5′-TAACACGAGGAGC-CGGGTACCGCACCCAAAGTACTGGGT-3′ were used to change Glu-42 and Pro-107 of 2B4 TCR Vβ to cysteines, respectively. The oligonucleotide 5′-CTGGTACACAGCAGGTTCTGGGTGAATTCATTAT-GGTATAACACTCAGAACGGT-3′ was used to introduce stop codons followed by an EcoRI site at the 3′ end of the Vα gene and the oligonucleotide 5′-CTGAATGACTTTTGAATTCATATGTCCACCCCCTGAGCC-ACC-3′ to introduce an NdeI site and ATG translation-initiation codon at the N terminus of Vβ. Restriction sites were introduced into these oligonucleotides to facilitate identification of mutated clones or subcloning.

The expression plasmid pYR-TCR2 for TCR(Vα-Cys) was generated by deletion of a Vβ–PE38-encoding EcoRI fragment. pYR-TCR3 encoding TCR(Vβ-Cys)–PE38 was constructed by deletion of a Vα-encoding NdeI fragment.

Expression, Refolding, and Purification

The components of TCR(dsFv)–PE38, TCR(Vα-Cys) and TCR(Vβ-Cys)–PE38 were produced in separate E. coli BL21 λDE3 cultures containing the corresponding plasmid. Upon induction with isopropyl-β-D-thiogalactoside, the recombinant proteins accumulate in intracellular inclusion bodies. The IBs were isolated, solubilized, refolded, and purified as described for the preparation of antibody dsFvs (Buchner et al., 1992; Brinkmann et al., 1993).

Binding of TCR-Specific MAbs

The fusion protein were immobilized on 96-well microtiter plates, blocked with 1% bovine serum albumin in PBS and then incubated with TCR-specific MAbs. The binding was detected by secondary alkaline phosphatase–labeled antibody as described (Kurucz et al., 1993). The 2B4 TCR contains Vα11 and Vβ3 allotypic determinants (Kurucz et al., 1993).

Cellular Inhibition Assays

Irradiated (2400 rads) spleen cells (5×10^5) from B10.A mice were incubated for 24 hr at 37°C with the 2B4 T cell hybridoma (2×10^4) and with different concentrations of the antigenic peptide in the presence or absence of the TCR(Fv) constructs or anti-class II antibody. Assays were done in triplicate in 96-well flat-bottomed plates in a final volume of 200 μl. After incubation, collected supernatants were assayed for IL-2 production by mouse IL-2 ELISA kit Interset-2X (Genzyme, Incorporated, Cambridge, Massachusetts). Recombinant mouse IL-2 was used as a standard.

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